Primary Mouse Neuron Progenitor Cells

Commonly used acronym: *Mouse NPCs*

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Contact person

Roel Quintens

Organisation

Name of the organisation Belgian Nuclear Research Centre

Department Interdisciplinary Biosciences

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Mouse
Type of cells/tissues/organs	Cortical neuron progenitor cells from E15 embryo

DESCRIPTION

Method keywords

Neuron progenitor cells

NPC

mouse brain

Cortex

neuron differentiation

Scientific area keywords

neurodevelopment

neurodevelopmental disorders

neuroscience

neurobiology

cellular proliferation

Method description

We have developed a protocol for culturing primary neuron progenitor cells (NPCs) derived from mouse embryos at embryonic day 15 (E15). These cells are highly proliferative, can be subcultured and cryopreserved and can be differentiated to neurons or astrocytes. Hence, this method can greatly reduce the number of laboratory animals needed for the culture of primary neuronal cultures. They can be grown as adherent monolayers or as neurospheres.
The protocol consist of the following steps: Dissection of brain from mouse fetus at E15, separation of dorsal forebrain, dissociation into single cell suspension, culture onto poly-D-lysin coated vessels (for adherent cultures), subculturing, cryopreservation of cells, thawing of frozen cells, differentiation to neurons, maturation of differentiated neurons. The protocol is partially based on the protocol first described by Steven Pollard, Methods Mol Biol 2013.

Lab equipment

- Dissection material
- Stereotactic microscope
- Horizontal flow cabinet
- Laminar flow cabinet
- Incubator

Method status

Still in development

History of use

Internally validated

PROS, CONS & FUTURE POTENTIAL

Advantages

Possibility of subculturing and cryopreservation constitutes an almost unlimited source of primary cells that can be used for differentiation into neurons, thereby dramatically reducing the need for animals to generate primary neuron cultures. The cells are highly suitable for research on neurodevelopmental diseases which most often underlie defects in neuron progenitors, rather than neurons. The cells are also very suitable for genetic interference, e.g. via siRNA, CRISPR-Cas9 or lentiviral vectors.

Challenges

The cells need specific growth media, which regularly needs to be prepared freshly and the medium should be changed every two days.

Modifications

We have started from the method as described by Pollard, Methods Mol Biol 2013 which did not work for us. We have gradually tweaked this method and are still trying to improve it by making small modifications, although our current protocol is very robust.

Future & Other applications

We currently have different cryopreserved cell lines from transgenic animals (e.g. Trp53 knockout) and cell lines that stably overexpress some of our genes of interest. Our research focuses on radiation-induced microcephaly but these cells could be applied as well for other neurodevelopmental disorders.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Mfossa et al., bioRxiv. 2020. doi: https://doi.org/10.1101/2020.06.26.171132. Not yet peer-reviewed.

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